

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

SEQ ID NO:3: Sense primer designed for preparation of SpaA and Δ SpaA protein by PCR amplification

Sequence length: 37 base pairs, which corresponds to the sequence of from the 79th to 106th nucleotide residues in SEQ ID NO:1

SEQ ID NO:4: Antisense primer designed for preparation of Δ SpaA protein by PCR amplification

Sequence length: 33 base pairs, which corresponds to the sequence of from the 1240th to 1260th nucleotide residues in SEQ ID NO:1

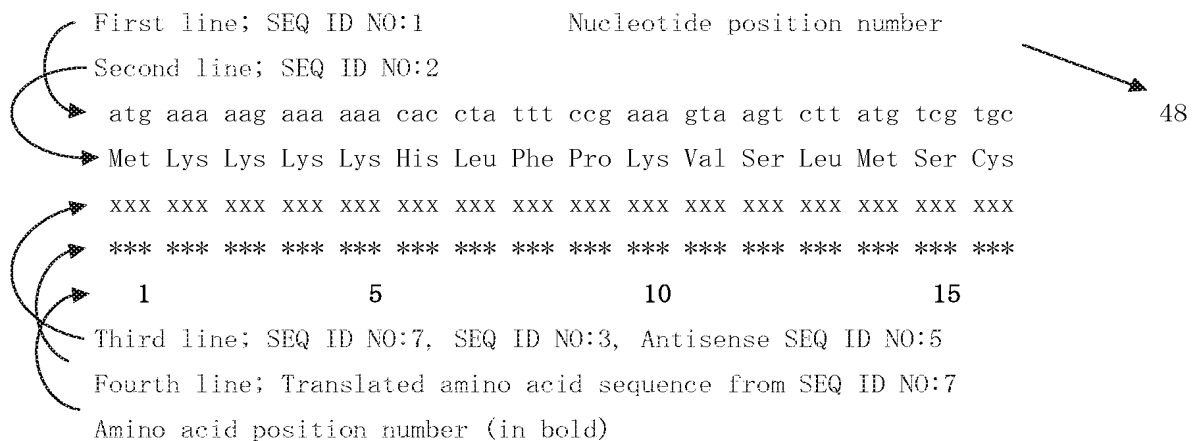
SEQ ID NO:5: Antisense primer designed for preparation of SpaA protein by PCR amplification

Sequence length: 38 base pairs, which corresponds to the sequence of from the 1855th to 1881st nucleotide residues in SEQ ID NO:1

SEQ ID NO:7: *Erysipelothrix rhusiopathiae* spaA gene, partial cds, strain:SE-9

Sequence length: 1748 base pairs, which corresponds to the sequence of from the 107th to 1854th nucleotide residues in SEQ ID NO:1

Expression of Spa A protein of SE-9 strain



The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

Expression of Spa A protein of SE-9 strain

1
atg aaa aag aaa aaa cac cta ttt ccg aaa gta agt ctt atg tcg tgc 48
Met Lys Lys Lys Lys His Leu Phe Pro Lys Val Ser Leu Met Ser Cys
xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx xxx
*** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

tta ctt tta aca gca atg cca cta caa aca gct ttt gct gat tcg aca 96
Leu Leu Leu Thr Ala Met Pro Leu Gln Thr Ala Phe Ala Asp Ser Thr
70 79
|→ (NcoI site) Sequence ID#3
xxx xxx xxx xxx xxx xxx xxx cat gcc atg gct ttc gct gat tcg aca
*** *** *** *** *** *** *** *** *** Met Ala Phe Ala Asp Ser Thr
20 25 30

gat att tct gtg att cca cta atc ggt gaa caa gtt gga ttg ctc cca 144
Asp Ile Ser Val Ile Pro Leu Ile Gly Gln Gln Val Gly Leu Leu Pro
107
→ | → Sequence ID#7
gat att tct gtg att cca cta atc ggt gaa caa gtt gga ttg ctc cca
Asp Ile Ser Val Ile Pro Leu Ile Gly Gln Gln Val Gly Leu Leu Pro
35 40 45

gtt tta cct ggg aca ggg gta cat gct cag gaa tac aac aaa atg act 192
Val Leu Pro Gly Thr Glu Val Ile Ala Gln Gln Tyr Asp Lys Ser Thr
gtt tta cct ggg aca ggg ata cat gct cag gaa tac aac aaa atg act
Val Leu Pro Gly Thr Glu Ile Ile Ala Gln Gln Tyr Asp Lys Ser Thr
50 55 60

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gat gct tat att gaa aaa ttg gta tct cta att aat caa aaa gtg aag 240

Asp Ala Tyr Ile Glu Lys Leu Val Ser Leu Ile Asn Gln Lys Val Lys

206(a→g)

gat gct tat att gaa aat ttg gta tct cta att aat caa aaa gtg aag

Asp Ala Tyr Ile Glu Asn Leu Val Ser Leu Ile Asn Gln Lys Val Lys

Gly

65

70

75

80

ccg ttt ctt ata aat gag cca aag ggg tac caa agt ttc gaa gca gtg 288

Pro Phe Leu Ile Asn Gln Pro Lys Gly Tyr Gln Ser Phe Gln Ala Val

ccg ttt ctt ata aat gaa cca aag ggg tac caa agt ttc gaa gca gtg

Pro Phe Leu Ile Asn Gln Pro Lys Gly Tyr Gln Ser Phe Gln Ala Val

85

90

95

aat gaa gag att aac tcg att gta agt gaa ctt aaa aat gaa gga atg 336

Asn Gln Gln Ile Asn Ser Ile Val Ser Gln Leu Lys Asn Gln Gly Met

aat gaa gag att aac tcg att gta agt gaa ctt aaa cat gaa gga atg

Asn Gln Gln Ile Asn Ser Ile Val Ser Gln Leu Lys His Gln Gly Met

100

105

110

agt ctt caa aac att cac cat atg ttt aaa caa agc atc caa aac cta 384

Ser Leu Gln Asn Ile His His Met Phe Lys Gln Ser Ile Gln Asp Leu

agt ctt caa aac att cac cat atg ttt aaa caa agc atc caa aac cta

Ser Leu Gln Asn Ile His His Met Phe Lys Gln Ser Ile Gln Asp Leu

115

120

125

gca act aga atc ggc tac aga agt ttt atg cag gat gct atg tat ctt 432

Ala Thr Arg Ile Gly Tyr Arg Ser Phe Met Gln Asn Ala Met Tyr Leu

gca act aga atc ggc tac aga agt ttt atg cag gat gct atg tat ctt

Ala Thr Arg Ile Gly Tyr Arg Ser Phe Met Gln Asn Ala Met Tyr Leu

130

135

140

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

gaa aat ttt gaa aga tta acg att cct gaa ctt gat gaa gca tac gtt 480

Glu Asn Phe Glu Arg Leu Thr Ile Pro Glu Leu Asp Glu Ala Tyr Val

461(a→g)

gaa aat ttt gaa aga tta acg att cct gaa ctt gat gaa gca tac gtt

Glu Asn Phe Glu Arg Leu Thr Ile Pro Glu Leu Asp Glu Ala Tyr Val

Gly

145

150

155

160

gat tta ctc gtg aat tac gag gtg aaa cac cgt att tta gta aaa tat 528

Asp Leu Leu Val Asn Tyr Glu Val Lys His Arg Ile Leu Val Lys Pro

gat tta ctc gtg aat tac gag gtg aaa cac cgt att tta gta aaa tat

Asp Leu Leu Val Asn Tyr Glu Val Lys His Arg Ile Leu Val Lys Pro

165

170

175

gaa ggt aaa gtt aaa ggt aga gct ccc tta gaa gca ttt ata gtt cct 576

Glu Gly Lys Val Lys Glu Arg Ala Pro Leu Glu Ala Phe Ile Val Pro

gaa gat aaa gtt aaa ggt aga gct cca tta gaa gca ttt ata gtt cct

Glu Asp Lys Val Lys Glu Arg Ala Pro Leu Glu Ala Phe Ile Val Pro

180

185

190

cta aga gat aga att cgt agt atg aat gaa att gct gca gaa gta aat 624

Leu Arg Asp Arg Ile Arg Ser Met Asn Glu Ile Ala Ala Glu Val Asn

608(t→c)

cta aga aat aga att cgt agt atg aat gaa att gct gca gaa gta aat

Leu Arg Asn Arg Ile Arg Ser Met Asn Glu Ile Ala Ala Glu Val Asn

Thr

195

200

205

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

tat tta cct gaa gcg cat gag gat ttc tta gtt tca gat tca agc gag 672

Tyr Leu Pro Glu Ala His Glu Asp Phe Leu Val Ser Asp Ser Ser Glu

642(t→g)

tat tta cct gaa gcg cat gag gat ttc tta gtt tca gat tca agc gag

Tyr Leu Pro Glu Ala His Glu Asp Phe Leu Val Ser Asp Ser Ser Glu

Gln

210

215

220

tat aat gac aaa cta aat aat atc aac ttt gct ttg ggt cta ggg gtc 720

Tyr Asn Asp Lys Leu Asn Asn Ile Asn Phe Ala Leu Gly Leu Gly Val

tat aat gac aaa cta aat aat atc aac ttt gct ttg ggt cta ggg gtc

Tyr Asn Asp Lys Leu Asn Asn Ile Asn Phe Ala Leu Gly Leu Gly Val

225

230

235

240

agc gag ttt att gac tat aac cgg ctc gaa aat atg atg gaa aaa gaa 768

Ser Glu Phe Ile Asp Tyr Asn Arg Leu Glu Asn Met Met Glu Lys Glu

758(t→c)

agc gag ttt att gac tat aac cgg ctc gaa aat atg atg gaa aaa gaa

Ser Glu Phe Ile Asp Tyr Asn Arg Leu Glu Asn Met Met Glu Lys Glu

Thr

245

250

255

ctt cat cca ctg tat ctt gaa ctt tat gct atg cgg aga aat cgc caa 816

Leu His Pro Leu Tyr Leu Glu Leu Tyr Ala Met Arg Arg Asn Arg Glu

att cat cca ttg tat ctt gaa ctt tat gct atg cgg aga aat cgc caa

Ile His Pro Leu Tyr Leu Glu Leu Tyr Ala Met Arg Arg Asn Arg Glu

260

265

270

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

att caa gtt gta aga gat gta tat cca aac ttg gaa cgt gcg aac gcg 864

Ile Gln Val Val Arg Asp Val Tyr Pro Asn Leu Gln Arg Ala Asn Ala

333(a→g)

att caa gtt gta aga gat gta tat cca aac ttg gaa cgt gcg aac gcg

Ile Gln Val Val Arg Asp Val Tyr Pro Asn Leu Gln Arg Ala Asn Ala

Gly

275

280

285

gtt gtt gaa tcc tta aag aca att aaa gat ata aaa caa aga ggg aag 912

Val Val Gln Ser Leu Lys Thr Thr Tyr Asp Thr Lys Gln Arg Gly

gtt gtt gaa tcc tta aag aca att aaa gat ata aaa caa aga gag aag

Val Val Gln Ser Leu Lys Thr Thr Lys Asp Thr Lys Gln Arg Glu

290

295

300

aaa cta cag gaa ctt ctt gaa att tat atc caa aga agt gga gat gtt 960

Lys Leu Gln Gln Leu Leu Gln Thr Tyr Thr Gln Arg Ser Gly Asp Val

aaa cta cag gaa ctt ctt gaa att tat atc caa aga agt gga gat gtt

Lys Leu Gln Gln Leu Leu Gln Thr Tyr Thr Gln Arg Ser Gly Asp Val

305

310

315

320

cga aaa cca gat gta ctc caa cga ttt att gga aaa tat caa tca gta 1008

Arg Lys Pro Asn Val Leu Gln Arg Phe Thr Gly Lys Tyr Gln Ser Val

cga aaa cca gat gta ctc caa cga ttt att gga aaa tat caa tca gta

Arg Lys Pro Asn Val Leu Gln Arg Phe Thr Gly Lys Tyr Gln Ser Val

325

330

335

gtt gat gaa gaa aaa aat aaa ctt caa gat tat tta gaa tca gat att 1056

Val Asn Gln Gln Lys Asn Lys Leu Gln Asp Tyr Leu Gln Ser Asp Thr

gtt gat gaa gaa aaa aat aaa ctt caa gat tat tta gaa tca gat att

Val Asn Gln Gln Lys Asn Lys Leu Gln Asp Tyr Leu Gln Ser Asp Thr

340

345

350

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

ttt gat tca tat agt gtg gat ggc gag aaa ata aga aat aaa gaa att 1104
Phe Asp Ser Tyr Ser Val Asp Gly Glu Lys Ile Arg Asp Lys Glu Ile
ttt gat tca tat agt gtg gat ggc gag aaa ata aga aat aaa gaa att
Phe Asp Ser Tyr Ser Val Asp Gly Glu Lys Ile Arg Asp Lys Glu Ile
355 360 365

aca ctc atc aat aga gat gca tac tta tct atg att tac aga gct caa 1152
Thr Leu Ile Asp Arg Asp Ala Tyr Leu Ser Met Ile Tyr Arg Ala Glu
aca ctc atc aat aga gat gca tac tta tct atg att tac aga gct caa
Thr Leu Ile Asp Arg Asp Ala Tyr Leu Ser Met Ile Tyr Arg Ala Glu
370 375 380

tcg att tcg gaa att aag acg att cgt gca gat tta gaa tca ctt gtc 1200
Ser Ile Ser Glu Ile Lys Thr Ile Arg Ala Asp Leu Glu Ser Leu Val
tcg att tcg gaa att aag acg att cgt gca gat tta gaa tca ctt gtc
Ser Ile Ser Glu Ile Lys Thr Ile Arg Ala Asp Leu Glu Ser Leu Val
385 390 395 400

1240
| ← Antisense
cct gaa agt

aaa tca ttc caa aat gaa gaa agt gac tct aaa gta gag cct gaa agt 1248
Lys Ser Phe Glu Asp Glu Glu Ser Asp Ser Lys Val Glu Pro Glu Ser
aaa tca ttc caa aat gaa gaa agt gat tct aaa gta gag cct gaa agt
Lys Ser Phe Glu Asp Glu Glu Ser Asp Ser Lys Val Glu Pro Glu Ser
405 410 415

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1260

Sequence ID#4 | (BamHISite)

ccc gtt aaa gta taa gga tcc gcg

Stop

ccc gtt aaa gta gaa aaa cca gtt gat gaa gaa aaa cct aaa gat caa 1296

Pro Val Lys Val Glu Lys Pro Val Asp Glu Val Lys Pro Lys Asp Glu

ccc gtt aaa gta gaa aaa cca gtt gat aaa gaa aaa cct aaa gat caa

Pro Val Lys Val Glu Lys Pro Val Asp Lys Val Lys Pro Lys Asp Glu

420 425 430

aag aag cta gtt gat caa tca aaa ccc gaa tgg aat tca aaa gaa ggg 1344

Lys Lys Leu Val Asp Glu Ser Lys Pro Glu Ser Asp Ser Lys Glu Glu

aag aag cca gtt gat caa tca aaa ccc gaa tgg aat tca aaa gaa ggg

Lys Lys Pro Val Asp Glu Ser Lys Pro Glu Ser Asp Ser Lys Glu Glu

435 440 445

tgg att aag aaa gat aat aag tgg ttc tat att gag aaa tca ggt gga 1392

Trp Ile Lys Lys Asp Asn Lys Trp Phe Tyr Ile Glu Lys Ser Gly Gly

tgg att aag aaa gat aat aag tgg ttc tat att gag aaa tca ggt gga

Trp Ile Lys Lys Asp Asn Lys Trp Phe Tyr Ile Glu Lys Ser Gly Gly

450 455 460

atg gca aca ggt tgg aag aag gta gca gac aaa tgg tac tac ctc gat 1440

Met Ala Thr Gly Trp Lys Lys Val Ala Asp Lys Trp Tyr Tyr Leu Asp

atg gca aca gga tgg aag aag gta gga gac aaa tgg tac tac ctc gat

Met Ala Thr Gly Trp Lys Lys Val Gly Asp Lys Trp Tyr Tyr Leu Asp

465 470 475 480

The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

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aat acg ggt gct ata gtt acg ggt tgg aag aag gta gca aac aaa tgg      1488
Asn Thr Gly Ala Ile Val Thr Gly Trp Lys Lys Val Ala Asn Lys Trp
aat acg ggt gct atg gtt acg ggt tgg aag aag gta gca aac aaa tgg
Asn Thr Gly Ala Met Val Thr Gly Trp Lys Lys Val Ala Asn Lys Trp
                                485                490                495

tac tat ctt gaa aaa tca ggt gcg atg gca aca gga tgg aag aaa gta      1536
Trp Tyr Leu Glu Lys Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Val
tac tac ctt gaa aac tca ggt gcg atg gca aca gga tgg aag aaa gta
Trp Tyr Leu Glu Asn Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Val
                                500                505                510

tca aac aag tgg tac tac ctt gaa aac tca ggt gca atg gca aca gga      1584
Ser Asn Lys Trp Tyr Tyr Leu Glu Asn Ser Gly Ala Met Ala Thr Gly
tca aac aag tgg tac tac ctt gaa aac tca ggt gcg atg gca aca gga
Ser Asn Lys Trp Tyr Tyr Leu Glu Asn Ser Gly Ala Met Ala Thr Gly
                                515                520                525

tgg aag aaa gta tca aac aag tgg tac tac ctt gaa aat tca ggc gca      1632
Trp Lys Lys Val Ser Asn Lys Trp Tyr Tyr Tyr Leu Glu Asn Ser Gly Ala
1591(a→g)
tgg aag aga gta tca aac aag tgg tac tac ctt gaa aat tca ggc gca
Trp Lys Arg Val Ser Asn Lys Trp Tyr Tyr Tyr Leu Glu Asn Ser Gly Ala
Gly
                                530                535                540

atg gct aca gga tgg aaa aag gta gca aac aaa tgg tac tac ctt gaa      1680
Met Ala Thr Gly Trp Lys Lys Val Ala Asn Lys Trp Tyr Tyr Tyr Leu Glu
atg gct aca gga tgg aaa aag gta gca aac aaa tgg tac tac ctt gaa
Met Ala Thr Gly Trp Lys Lys Val Ala Asn Lys Trp Tyr Tyr Tyr Leu Glu
                                545                550                555                560

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The spaA gene cloning of SE-9 strain by using PCR primer designed from the spaA gene sequence (SEQ ID#1) of Fujisawa strain

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aac tca ggt gcg atg gca aca gga tgg aag aaa gta tcg aac aag tgg      1728
Leu Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Val Ser Asn Lys Trp
aac tca ggt gcg atg gca aca gga tgg aag aaa gta tcg aac aag tgg
Asn Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Val Ser Asn Lys Trp
                                     565           570           575

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tac tac ctt gaa aac tca ggc gca atg gct aca gga tgg aaa aag gta      1776
Tyr Tyr Leu Glu Asn Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Val
tac tac ctt gaa aac tca ggc gca atg gca acg ggt tgg aag aaa ata
Tyr Tyr Leu Glu Asn Ser Gly Ala Met Ala Thr Gly Trp Lys Lys Ile
                                     580           585           590

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gca aac aaa tgg tac tac ctt gat aaa tca gga atg atg gtt aca ggt      1824
Ala Asn Lys Trp Tyr Tyr Leu Asp Lys Ser Gly Met Met Val Thr Glu
gca aat aaa tgg tac tac ctt gat aaa tca gga atg atg gtt aca ggt
Ala Asn Lys Trp Tyr Tyr Leu Asp Lys Ser Gly Met Met Val Thr Glu
                                     595           600           605

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tca aaa tct att gat ggt aaa aag tat gca ttt aag aac gat gga agt      1872
Ser Lys Ser Ile Asp Gly Lys Lys Tyr Ala Phe Lys Asn Asp Gly Ser
                                     1855

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Sequence ID#7	→		←	Antisense Sequence ID#5
tca aaa tct att gat ggt aaa aag tat gca				ttt aag aac gat gga agt
Ser Lys Ser Ile Asp Gly Lys Lys Tyr Ala				Phe Lys Asn Asp Gly Ser
610				620

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tta aaa tag      1881
Leu Lys Stop
1831
| (BamHISite)
tta aaa tag agg gat ccg cg
Leu Lys Stop
625           630

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